

What is claimed is:

1. A medium for sequentially detecting two or more bacterial enzymes, comprising:

(a) a first nutrient indicator which provides a first detectable signal when cleaved by a first bacterial enzyme; and

(b) a second nutrient indicator which provides an intermediate molecule when cleaved by a second bacterial enzyme, wherein said intermediate molecule provides a second detectable signal upon reacting with a developing agent.

2. The medium of claim 1, wherein said first bacterial enzyme is  $\beta$ -glucosidase and said first nutrient indicator produces a detectable color when cleaved by  $\beta$ -glucosidase.

3. The medium of claim 2, wherein said first nutrient indicator is selected from the group consisting of resofuran- $\beta$ -D-glucopyranoside, o-nitrophenyl- $\beta$ -D-glucopyranoside, p-nitrophenyl- $\beta$ -D-glucopyranoside, 5-bromo-4-chloro-3-indoxyl- $\beta$ -D-glucopyranoside, 6-bromo-2-naphtyl- $\beta$ -D-glucopyranoside, Rose- $\beta$ -D-glucopyranoside, VQM-Glc(2-{2-[4-( $\beta$ -D-glucopyranosyloxy)-3-methoxyl]vinyl}-1-methyl-quinolinium iodide, VBZTM-Gluc(2-{2-[4-( $\beta$ -D-glucopyranosyloxy)3-methoxyphenyl]vinyl}-3-

methybenzothiazolium iodide, and 4-methylumbelliferyl- $\beta$ -D-glucopyranoside.

4. The medium of claim 2, wherein said first nutrient indicator is o-nitrophenyl- $\beta$ -D-glucopyranoside.

5. The medium of claim 1, wherein said second enzyme is pyrrolidonyl arylamidase and said intermediate molecule alters the color of said medium upon reacting with a color developing agent.

6. The medium of claim 5, wherein said second nutrient indicator is pyroglutamic acid- $\beta$ -naphthylamide.

7. The medium of claim 5, wherein said color developing agent is p-dimethylaminocinnamaldehyde.

8. A method for sequentially detecting the presence of two or more bacterial enzymes in a sample, comprising the steps of:

(a) inoculating any medium of claims 1-6 with said sample and incubating the medium under conditions suitable for bacterial growth;

(b) observing said first detectable signal, the presence of which indicates said sample containing said first bacterial enzyme; and

(c) contacting said medium or a portion of said medium with said developing agent and observing said second detectable signal, the presence of which indicates said sample containing said second enzyme.

9. The method of claim 8, wherein a portion of said medium is brought into contact with said developing agent on a filter paper or an absorbant material and observing said second detectable signal, the presence of which indicates said sample containing said second enzyme.

10. A medium for detecting the presence or absence of vancomycin-resistant *Enterococci*, comprising:

- a) vancomycin in an amount sufficient to suppress the growth of vancomycin sensitive *Enterococci*;
- b) one or more selective agents in an amount sufficient to suppress the growth of fungi, gram positive and gram negative bacteria other than *Enterococci*;
- c) a first nutrient indicator which provides a first detectable signal when cleaved by  $\beta$ -glucosidase; and
- d) a second nutrient indicator which provides an intermediate molecule when cleaved by pyrrolidonyl arylamidase, wherein said intermediate molecule provides a second detectable signal upon reacting with a developing agent.

11. The medium of claim 10 wherein said first nutrient indicator is selected from the group consisting of resofuran- $\beta$ -D-glucopyranoside, o-nitrophenyl- $\beta$ -D-glucopyranoside, p-nitrophenyl- $\beta$ -D-glucopyranoside, 5-bromo-4-chloro-3-indoxyl- $\beta$ -D-glucopyranoside, 6-bromo-2-naphtyl- $\beta$ -D-glucopyranoside, Rose- $\beta$ -D-glucopyranoside, VQM-Glc(2-{2-[4-( $\beta$ -D-glucopyranosyloxy)-3-methoxyl]vinyl}-1-methyl-quinolinium iodide, VBZTM-Gluc(2-{2-[4-( $\beta$ -D-glucopyranosyloxy)3-methoxylphenyl]vinyl}-3-methylbenzothiazolium iodide, and 4-methylumbelliferyl- $\beta$ -D-glucopyranoside.

12. The medium of claim 10, wherein said first nutrient indicator is o-nitrophenyl- $\beta$ -D-glucopyranoside.

13. The medium of claim 10, wherein said second nutrient indicator is pyroglutamic acid- $\beta$ -naphtylamide.

14. The medium of claim 13, wherein said developing agent is p-dimethylaminocinnamaldehyde.

15. The medium of claim 10, wherein said one or more selective agents are selected from the group consisting of amikacin sulfate, polymyxin B, bacitracin, clindamycin, cefotaxime, and amphotericin B.

16. The medium of claim 10, further comprising a test sample from a human source.

17. The medium of claim 16, wherein said test sample is a rectal or perirectal swab.

18. The medium of claim 16, wherein said test sample is a wound swab.

19. The medium of claim 16, wherein said test sample is a urine specimen.

20. The medium of claim 16, wherein said test sample is a stool specimen.

21. The medium of claim 16, wherein said test sample is a swab taken from a surface of utensil or equipment from a hospital.

22. The medium of claim 10 which is liquid.

23. A method for detecting the presence or absence of vancomycin-resistant *Enterococci* in a sample, comprising the steps of:

(a) inoculating any medium of claims 10-12 with said sample and incubating the medium under conditions suitable for the growth of vancomycin resistant *Enterococci*;

(b) observing said first detectable signal; and

(c) contacting said medium or a portion of said medium with said developing agent and observing said second detectable signal, wherein the presence of both said first detectable signal and said second detectable signal indicates said sample containing vancomycin-resistant *Enterococci*.

24. The method of claim 23, wherein a portion of said medium is brought into contact with said developing agent on a filter paper or an absorbant material and observing said second detectable signal, the presence of which indicates said sample containing vancomycin-resistant *Enterococci*.

25. The method of claim 23, wherein said first nutrient indicator is o-nitrophenyl- $\beta$ -D-glucopyranoside, said second nutrient indicator is pyroglutamic acid- $\beta$ -naphthylamide, and said developing agent is p-dimethylaminocinnamaldehyde.

26. The method of claim 23, wherein said medium is liquefied and said contacting in step (b) comprising taking a portion of said medium to mix with said developing agent.

27. The method of claim 23, wherein said medium is agar and said contacting in step (b) comprising adding said developing agent on top of said medium.

28. The method of claim 23, wherein said vancomycin resistant *Enterococci* are detected in no more than 24 hours from inoculation.

29. The method of claim 23, wherein said vancomycin resistant *Enterococci* in a concentration of no less than 1 viable microbes per milliliter of said medium at inoculation are detected in no more than 24 hours from inoculation.

30. The method of claim 23, wherein said vancomycin resistant *Enterococci* in a concentration of no less than 10 viable microbes per milliliter of said medium at inoculation are detected in no more than 18 hours from inoculation.

31. A medium for detecting the presence or absence of vancomycin-resistant *Enterococci*, comprising:

- a) vancomycin in an amount sufficient to suppress the growth of vancomycin sensitive *Enterococci*;
- b) one or more selective agents in an amount sufficient to suppress the growth of fungi, gram positive and gram negative bacteria other than *Enterococci*;
- c) a first nutrient indicator which provides a first detectable signal when cleaved by  $\beta$ -glucosidase; and
- d) a second nutrient indicator which provides a second detectable signal when cleaved by pyrrolidonyl arylamidase, wherein the presence of both said first

detectable signal and second detectable signal is  
distinctively detectable from the presence of only one of  
said detectable signals.

5           32. The medium of claim 31, wherein said first  
nutrient indicator is o-nitrophenyl- $\beta$ -D-glucopyranoside and  
said second nutrient indicator is L-pyroglutamic acid 7-  
amido-4-methyl-coumarin.

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